

STATE OF MISSOURI  
**DEPARTMENT OF NATURAL RESOURCES**

MISSOURI CLEAN WATER COMMISSION



**MISSOURI STATE OPERATING PERMIT**

In compliance with the Missouri Clean Water Law, (Chapter 644 R.S. Mo. as amended, hereinafter, the Law), and the Federal Water Pollution Control Act (Public Law 92-500, 92<sup>nd</sup> Congress) as amended,

Permit No. MO-0132489

Owner: Missouri Ethanol, LLC  
Address: 6158 Highway 54, P.O Box 99, Laddonia, MO 63352

Continuing Authority: same as owner  
Address: same as owner

Facility Name: Missouri Ethanol, LLC  
Facility Address: 6158 Highway 54, Laddonia, MO 63352

Legal Description: NE ¼, NW ¼, Sec. 36, T52N, R7W, Audrain County

Receiving Stream: Unnamed Tributary to Lick Creek (U)  
First Classified Stream and ID: Lick Creek (C) (0150)  
USGS Basin & Sub-watershed No.: 07110007-010001

is authorized to discharge from the facility described herein, in accordance with the effluent limitations and monitoring requirements as set forth herein:

**FACILITY DESCRIPTION**

Outfall #001 - Ethanol for Fuel Manufacturing SIC #2869

See page 2

This permit authorizes only wastewater discharges under the Missouri Clean Water Law and the National Pollutant Discharge Elimination System; it does not apply to other regulated areas. This permit may be appealed in accordance with Section 644.051.6 of the Law.

September 1, 2006

Effective Date

A handwritten signature in black ink, reading "Doyle Childers".

Doyle Childers, Director, Department of Natural Resources  
Executive Secretary, Clean Water Commission

August 31, 2011

Expiration Date  
MO 780-0041 (10-93)

G. Irene Crawford, Director, Northeast Regional Office

FACILITY DESCRIPTION (continued)

Outfall #001 - Cooling Tower Blow Down, upstream in pipe of outfall #007  
Design flow is 0.265 MGD.

Outfall #002 – Reverse Osmosis Reject Water, upstream in pipe of outfall #007  
Design Flow is 0.143 MGD

Outfall #003 – Cooling Tower Filter Backwash/Softening Regeneration, upstream in pipe of outfall #007  
Design Flow is 0.003 MGD

Outfall #004 – Storm Water Discharge – Plant Site Basin  
Storm water collection basin/sludge retained in basin/storm water runoff.  
Design flow 8.0 MGD (Peak discharge of 12.41 cfs in a 100 year 24 hour rain event)

Outfall #005 – Storm Water Discharge – North of Rail Spur Basin  
Storm water collection basin/sludge retained in basin/storm water runoff.  
Design flow 1.396 MGD (Peak discharge of 2.16 cfs in a 10 year-24 hour rain event)

Outfall #006 – Greensand Water Treatment Filter Backwash  
Water treatment basin/sludge retained in basin  
Design flow is 0.057 MGD

Outfall #007 – Combined flows from outfalls #001, #002, and #003.  
Design flow is 0.411 MGD

Outfall #008 – Combined flows from outfall #006 and outfall #007.  
Design flow is 0.468 MGD

<b>A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS</b>					PAGE NUMBER 3 of 11	
					PERMIT NUMBER MO-0132489	
The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:						
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
<u>Outfall #001</u> Cooling Tower Blow Down, upstream in pipe of outfall #007  Flow  Temperature  Conductivity  Chlorides	MGD  °F  µmho/cm @ 25 °C  mg/L	*  *  *  *		*  *  *  *	once/month  once/month  once/month  once/month	24 hr. estimate  grab  grab  grab
<u>Outfall #002</u> Reverse Osmosis Reject Water, upstream in pipe of outfall #007  Flow  Conductivity	MGD  µmho/cm @ 25 °C	*  *		*  *	once/month  once/month	24 hr. estimate  grab
<u>Outfall #003</u> Cooling Tower Filter Backwash/Softening Regeneration, upstream in pipe of outfall #007  Flow  Conductivity	MGD  µmho/cm @ 25 °C	*  *		*  *	once/month  once/month	24 hr. estimate  grab
MONITORING REPORTS SHALL BE SUBMITTED MONTHLY THE FIRST REPORT IS DUE <u>October 28, 2006</u> . THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.						
<b>B. STANDARD CONDITIONS</b>						
IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED <u>Parts I</u> STANDARD CONDITIONS DATED <u>October 1, 1980</u> AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.						

<b>A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS</b>					PAGE NUMBER 4 of 11	
					PERMIT NUMBER MO-0132489	
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OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
<u>Outfall #004 and Outfall #005- Storm Water Discharge - Plant Site Basin and North of Rail Spur Basin</u>  Flow  Biochemical Oxygen Demand <sub>5</sub>  Total Suspended Solids  Oil and Grease  pH - Units	MGD  mg/L  mg/L  mg/L  SU	*  45  50  15  **		*  30  50  10  **	once/month  once/month  once/month  once/month  once/month	24 hr. estimate  grab  grab  grab  grab
<u>Outfall #006 - Greensand Well Water Treatment System Backwash</u>  Flow  Settleable Solids  Total Residual Chlorine (Note 1)  Conductivity  pH	MGD  ML/L  mg/L  µmho/cm @ 25 °C  SU	*  1.0  0.019 (0.13)  *  **		*  1.0  0.019 (0.13)  *  **	once/month  once/month  once/month  once/month  once/month	24 hr. estimate  grab  grab  grab  grab
MONITORING REPORTS SHALL BE SUBMITTED MONTHLY THE FIRST REPORT IS DUE <u>October 28, 2006</u> . THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.						
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<b>A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS</b>					PAGE NUMBER 5 of 11	
					PERMIT NUMBER MO-0132489	
The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:						
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #007 Combined flows from outfalls #001, #002, and #003.						
Flow	MGD	*		*	once/month	24 hr. estimate
Biochemical Oxygen Demand <sub>5</sub>	mg/L	45		30	once/month	grab
Total Suspended Solids	mg/L	45		30	once/month	grab
Chlorides	mg/L	859		428	once/month	grab
Chlorides plus Sulfates	mg/L	1,000		1,000	once/month	grab
Oil and Grease	mg/L	15		10	once/month	grab
Ammonia as N	mg/L	12.1		6	once/month	grab
Total Residual Chlorine (Note 1)	mg/L	0.019 (0.13)		0.019 (0.13)	once/month	24 hr. estimate
Conductivity	µmho/cm @ 25 °C	*		*	once/month	grab
Temperature	°F	90		90	once/month	grab
pH - Units	SU	**		**	once/month	grab
MONITORING REPORTS SHALL BE SUBMITTED MONTHLY THE FIRST REPORT IS DUE <u>October 28, 2006</u> . THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.						
<b>B. STANDARD CONDITIONS</b>						
IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE <u>ATTACHED Parts I</u> <u>STANDARD CONDITIONS DATED October 1, 1980</u> AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.						

<b>A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS</b>				PAGE NUMBER 6 of 11		
				PERMIT NUMBER MO-0132489		
The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:						
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
		DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #008 Combined flows from outfall #006 and outfall #007						
Whole Effluent Toxicity (WET) Test	% Survival	See Special Condition #8			Once/year	24 hr. Composite
MONITORING REPORTS SHALL BE SUBMITTED <u>ANNUALLY</u> THE FIRST REPORT IS DUE <u>October 28, 2007</u> . THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.						
<b>B. STANDARD CONDITIONS</b>						
IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED <u>Parts I</u> STANDARD CONDITIONS DATED <u>October 1, 1980</u> AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.						

MO 780-0010 (8/91)

**A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS (continued)**

\* Monitoring requirement only.

\*\* pH is measured in pH units and is not to be averaged. The pH is limited to the range of 6.0-9.0 pH units.

Note 1: This effluent limit is below the minimum quantification level (ML) of the most common and practical EPA approved CLTRC methods. The department has determined the current acceptable ML for total residual chlorine to be 0.13 mg/L when using the DPD Colorimetric Method #4500 - CL G. from Standard Methods for the Examination of Waters and Wastewater. The permittee will conduct analyses in accordance with this method, or equivalent, and report actual analytical values. Measured values greater than or equal to the minimum quantification level of 0.13 mg/L will be considered violations of the permit and values less than the minimum quantification level of 0.13 mg/L will be considered to be in compliance with the permit limitation. The minimum quantification level does not authorize the discharge of chlorine in excess of the effluent limits stated in the permit.

**C. SPECIAL CONDITIONS**

1. This permit may be reopened and modified, or alternatively revoked and reissued, to:
  - (a) Comply with any applicable effluent standard or limitation issued or approved under Sections 301(b)(2)(C) and (D), 304(b)(2), and 307(a) (2) of the Clean Water Act, if the effluent standard or limitation so issued or approved:
    - (1) contains different conditions or is otherwise more stringent than any effluent limitation in the permit; or
    - (2) controls any pollutant not limited in the permit.
  - (b) Incorporate new or modified effluent limitations or other conditions, if the result of a waste load allocation study, toxicity test or other information indicates changes are necessary to assure compliance with Missouri's Water Quality Standards.
  - (c) Incorporate new or modified effluent limitations or other conditions if, as the result of a watershed analysis, a Total Maximum Daily Load (TMDL) limitation is developed for the receiving waters which are currently included in Missouri's list of waters of the state not fully achieving the state's water quality standards, also called the 303(d) list.

The permit as modified or reissued under this paragraph shall also contain any other requirements of the Clean Water Act then applicable.

C. SPECIAL CONDITIONS (continued)

2. All outfalls must be clearly marked in the field.

3. Permittee will cease discharge by connection to areawide wastewater treatment system within 90 days of notice of its availability.

4. Changes in Discharges of Toxic Substances

The permittee shall notify the Director as soon as it knows or has reason to believe:

- (a) That any activity has occurred or will occur which would result in the discharge of any toxic pollutant which is not limited in the permit, if that discharge will exceed the highest of the following "notification levels:"
  - (1) One hundred micrograms per liter (100 µg/L);
  - (2) Two hundred micrograms per liter (200 µg/L) for acrolein and acrylonitrile; five hundred micrograms per liter (500 µg/L) for 2,5 dinitrophenol and for 2-methyl-4, 6-dinitrophenol; and one milligram per liter (1 mg/L) for antimony;
  - (3) Five (5) times the maximum concentration value reported for the pollutant in the permit application;
  - (4) The level established in Part A of the permit by the Director.
- (b) That they have begun or expect to begin to use or manufacture as an intermediate or final product or byproduct any toxic pollutant, which was not reported in the permit application.

5. Report as no-discharge when a discharge does not occur during the report period.

6. General Criteria. The following water quality criteria shall be applicable to all waters of the state at all times including mixing zones. No water contaminant, by itself or in combination with other substances, shall prevent the waters of the state from meeting the following conditions:

- (a) Waters shall be free from substances in sufficient amounts to cause the formation of putrescent, unsightly or harmful bottom deposits or prevent full maintenance of beneficial uses;
- (b) Waters shall be free from oil, scum and floating debris in sufficient amounts to be unsightly or prevent full maintenance of beneficial uses;
- (c) Waters shall be free from substances in sufficient amounts to cause unsightly color or turbidity, offensive odor or prevent full maintenance of beneficial uses;
- (d) Waters shall be free from substances or conditions in sufficient amounts to result in toxicity to human, animal or aquatic life;
- (e) There shall be no significant human health hazard from incidental contact with the water;
- (f) There shall be no acute toxicity to livestock or wildlife watering;
- (g) Waters shall be free from physical, chemical or hydrologic changes that would impair the natural biological community;
- (h) Waters shall be free from used tires, car bodies, appliances, demolition debris, used vehicles or equipment and solid waste as defined in Missouri's Solid Waste Law, section 260.200, RSMo, except as the use of such materials is specifically permitted pursuant to section 260.200-260.247.

7. Sludge and Biosolids Use For Domestic Wastewater Treatment Facilities

- (a) Permittee shall comply with the pollutant limitations, monitoring, reporting, and other requirements in accordance with the attached permit Standard Conditions.
- (b) If sludge is not removed by a contract hauler, Permittee is authorized to land apply biosolids that are removed from the domestic wastewater treatment lagoon during lagoon clean-out and maintenance activities. Permit Standard Conditions, Part III shall apply to the land application of biosolids. Permittee shall notify the department at least 180 days prior to the planned removal of biosolids from the lagoon. The department may require submittal of a biosolids management plan for department review and approval as determined appropriate on a case-by-case basis.

8. Whole Effluent Toxicity (WET) tests shall be conducted as follows:

SUMMARY OF WET TESTING FOR THIS PERMIT				
OUTFALL	A.E.C. %	FREQUENCY	SAMPLE TYPE	MONTH
#008	100%	ANNUALLY	24 hr. Sample	July

(a) Test Schedule and Follow-Up Requirements

- (1) Perform a SINGLE-dilution test in the months and at the frequency specified above. For tests which are successfully passed, submit test results USING THE DEPARTMENT'S WET TEST REPORT FORM #MO-780-1899 along with complete copies of the test reports as received from the laboratory, including copies of chain-of-custody forms within 30 calendar days of availability to the WATER PROTECTION PROGRAM, P.O. Box 176, Jefferson City, MO 65102. If the effluent passes the test, do not repeat the test until the next test period.
  - (a) For discharges of stormwater, samples shall be collected within three hours from when discharge first occurs.
  - (b) Samples submitted for analysis of stormwater discharges shall be collected as a grab.
  - (c) For discharges of non-stormwater, samples shall be collected only when precipitation has not occurred for a period of forty-eight hours prior to sample collection. In no event shall sample collection occur simultaneously with the occurrence of precipitation excepting for stormwater samples.
  - (d) A twenty-four hour composite sample shall be submitted for analysis of non-stormwater discharges.
  - (e) Upstream receiving water samples, where required, shall be collected upstream from any influence of the effluent where downstream flow is clearly evident.
  - (f) Samples submitted for analysis of upstream receiving water may be collected as either a grab or twenty-four-hour composite as appropriate to the nature of the discharge.
  - (g) Chemical and physical analysis of the upstream control and effluent sample shall occur immediately upon being received by the laboratory, prior to any manipulation of the effluent sample beyond preservation methods consistent with federal guidelines for WET testing that are required to stabilize the sample during shipping.
  - (h) Any and all chemical or physical analysis of the effluent sample performed in conjunction with the WET test shall be performed at the 100% Effluent concentration in addition to analyses performed upon any other effluent concentration.
  - (i) All chemical analyses included in the Missouri Department of Natural Resources WET test report form #MO-780-1899 shall be performed and results shall be recorded in the appropriate field of the report form.
  - (j) Where flow-weighted composite sample is required for analysis, the samples shall be composited at the laboratory where the test is to be performed.
  - (k) Where in stream testing is required downstream from the discharge, sample collection shall occur immediately below the established Zone of Initial Dilution in conjunction with or immediately following a release or discharge.
  - (l) Samples submitted for analysis of downstream receiving water may be collected as either a grab or twenty-four-hour composite as appropriate to the nature of the discharge.



8. Whole Effluent Toxicity (continued)

- (m) All instream samples, including downstream samples, shall be tested for toxicity at the 100% concentration in addition to any other assigned AEC for in-stream samples.
  - (2) All failing test results along with complete copies of the test reports as received from the laboratory, INCLUDING THOSE TESTS CONDUCTED UNDER CONDITION (3) BELOW, shall be reported to the WATER PROTECTION PROGRAM, P.O. Box 176, Jefferson City, MO 65102 within 14 calendar days of the availability of the results.
  - (3) If the effluent fails the test, a multiple dilution test shall be performed within 30 calendar days and biweekly thereafter, until one of the following conditions are met:
    - (a) THREE CONSECUTIVE MULTIPLE-DILUTION TESTS PASS. No further tests need to be performed until next regularly scheduled test period.
    - (b) A TOTAL OF THREE MULTIPLE-DILUTION TESTS FAIL.
  - (4) Failure of at least two multiple-dilution tests during any period of accelerated monitoring violates the permit narrative requirement for aquatic life protection.
  - (5) The permittee shall submit a CONCISE summary of all test results for the test series to the WATER PROTECTION PROGRAM, P.O. Box 176, Jefferson City, MO 65102 within 14 calendar days of the third failed test.
  - (6) Additionally, the following shall apply upon failure of the third MULTIPLE DILUTION test: A toxicity identification evaluation (TIE) or toxicity reduction evaluation (TRE) is automatically triggered. The permittee shall contact THE WATER PROTECTION PROGRAM within 14 calendar days from availability of the test results to ascertain as to whether a TIE or TRE is appropriate. The permittee shall submit a plan for conducting a TIE or TRE to the WATER PROTECTION PROGRAM within 60 calendar days of the date of DNR's direction to perform either a TIE or TRE. This plan must be approved by DNR before the TIE or TRE is begun. A schedule for completing the TIE or TRE shall be established in the plan approval.
  - (7) Upon DNR's approval, the TIE/TRE schedule may be modified if toxicity is intermittent during the TIE/TRE investigations. A revised WET test schedule may be established by DNR for this period.
  - (8) If a previously completed TIE has clearly identified the cause of toxicity, additional TIEs will not be required as long as effluent characteristics remain essentially unchanged and the permittee is proceeding according to a DNR approved schedule to complete a TRE and reduce toxicity. Regularly scheduled WET testing as required in the permit, without the follow-up requirements, will be required during this period.
  - (9) When WET test sampling is required to run over one DMR period, each DMR report shall contain a copy of the Department's WET test report form that was generated during the reporting period.
  - (10) Submit a concise summary in tabular format of all test results with the annual report.
- (b) PASS/FAIL procedure and effluent limitations:
- (1) To pass a single-dilution test, mortality observed in the AEC test concentration shall not be significantly different (at the 95% confidence level;  $p = 0.05$ ) than that observed in the upstream receiving-water control sample. Where upstream receiving water is not available mortality observed in the AEC test concentration shall not be significantly different (at the 95% confidence level;  $p = 0.05$ ) than that observed in the laboratory control. The appropriate statistical tests of significance shall be consistent with the most current edition of METHODS FOR MEASURING THE ACUTE TOXICITY OF EFFLUENTS AND RECEIVING WATERS TO FRESHWATER AND MARINE ORGANISMS or other Federal guidelines as appropriate or required.

8. Whole Effluent Toxicity (continued)

- (2) To pass a multiple-dilution test:
  - (a) For facilities with a computed percent effluent at the edge of the zone of initial dilution, Allowable Effluent Concentration (AEC), OF 30% OR LESS THE AEC must be less than three-tenths (0.3) of the LC<sub>50</sub> concentration for the most sensitive of the test organisms; **OR**,
  - (b) For facilities with an AEC greater than 30% the LC<sub>50</sub> concentration must be greater than 100%; **AND**,
  - (c) all effluent concentrations equal to or less than the AEC must be nontoxic. Mortality observed in all effluent concentrations equal to or less than the AEC shall not be significantly different (at the 95% confidence level;  $p = 0.05$ ) than that observed in the upstream receiving-water control sample. Where upstream receiving water is not available mortality observed in the AEC test concentration shall not be significantly different (at the 95% confidence level;  $p = 0.05$ ) than that observed in the laboratory control. The appropriate statistical tests of significance shall be consistent with the most current edition of METHODS FOR MEASURING THE ACUTE TOXICITY OF EFFLUENTS AND RECEIVING WATERS TO FRESHWATER AND MARINE ORGANISMS or other federal guidelines as appropriate or required. Failure of one multiple-dilution test may be considered an effluent limit violation.

(c) Test Conditions

- (1) Test Type: Acute Static non-renewal
- (2) Test species: Ceriodaphnia dubia and Pimephales promelas (fathead minnow). Organisms used in WET testing shall come from cultures reared for the purpose of conducting toxicity tests and cultured in a manner consistent with the most current USEPA guidelines. All test animals shall be cultured as described in the most current edition of METHODS FOR MEASURING THE ACUTE TOXICITY OF EFFLUENTS AND RECEIVING WATERS TO FRESHWATER AND MARINE ORGANISMS.
- (3) Test period: 48 hours at the "Acceptable Effluent Concentration" (AEC) specified above.
- (4) When dilutions are required, upstream receiving stream water shall be used as dilution water. If upstream water is unavailable or if mortality in the upstream water exceeds 10%, "reconstituted" water will be used as dilution water. Procedures for generating reconstituted water will be supplied by the MDNR upon request.
- (5) Single-dilution tests will be run with:
  - (a) Effluent at the AEC concentration;
  - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
  - (c) reconstituted water.
- (6) Multiple-dilution tests will be run with:
  - (a) 100%, 50%, 25%, 12.5%, and 6.25% effluent, unless the AEC is less than 25% effluent, in which case dilutions will be 4 times the AEC, two times the AEC, AEC, 1/2 AEC and 1/4 AEC;
  - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
  - (c) reconstituted water.
- (7) If reconstituted-water control mortality for a test species exceeds 10%, the entire test will be rerun.
- (8) If upstream control mortality exceeds 10%, the entire test will be rerun using reconstituted water as the dilutant.

## SUMMARY OF TEST METHODOLOGY FOR WHOLE-EFFLUENT TOXICITY TESTS

Whole-effluent-toxicity test required in NPDES permits shall use the following test conditions when performing single or multiple dilution methods. Any future changes in methodology will be supplied to the permittee by the Missouri Department of Natural Resources (MDNR). Unless more stringent methods are specified by the DNR, the procedures shall be consistent with the most current edition of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.

### Test conditions for Ceriodaphnia dubia:

Test duration:	48 h
Temperature:	25 ± 1°C Temperatures shall not deviate by more than 3°C during the test.
Light Quality:	Ambient laboratory illumination
Photoperiod:	16 h light, 8 h dark
Size of test vessel:	30 mL (minimum)
Volume of test solution:	15 mL (minimum)
Age of test organisms:	<24 h old
No. of animals/test vessel:	5
No. of replicates/concentration:	4
No. of organisms/concentration:	20 (minimum)
Feeding regime:	None (feed prior to test)
Aeration:	None
Dilution water:	Upstream receiving water; if no upstream flow, synthetic water modified to reflect effluent hardness.
Endpoint:	Pass/Fail (Statistically significant Mortality when compared to upstream receiving water control or synthetic control if upstream water was not available at $p \leq 0.05$ )
Test acceptability criterion:	90% or greater survival in controls

### Test conditions for Pimephales promelas:

Test duration:	48 h
Temperature:	25 ± 1°C Temperatures shall not deviate by more than 3°C during the test.
Light Quality:	Ambient laboratory illumination
Photoperiod:	16 h light/ 8 h dark
Size of test vessel:	250 mL (minimum)
Volume of test solution:	200 mL (minimum)
Age of test organisms:	1-14 days (all same age)
No. of animals/test vessel:	10
No. of replicates/concentration:	4 (minimum) single dilution method 2 (minimum) multiple dilution method
No. of organisms/concentration:	40 (minimum) single dilution method 20 (minimum) multiple dilution method
Feeding regime:	None (feed prior to test)
Aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min.
Dilution water:	Upstream receiving water; if no upstream flow, synthetic water modified to reflect effluent hardness.
Endpoint:	Pass/Fail (Statistically significant Mortality when compared to upstream receiving water control or synthetic control if upstream water was not available at $p \leq 0.05$ )
Test Acceptability criterion:	90% or greater survival in controls